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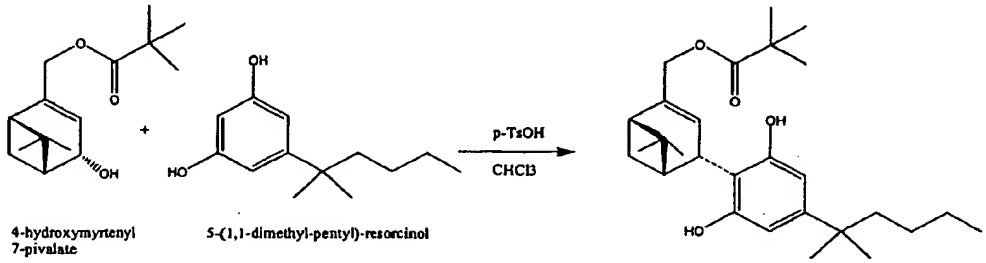
(54) Title: BICYCLIC CB2 CANNABINOID RECEPTOR LIGANDS

(57) Abstract: The present invention relates to non-classical cannabinoids that are ligands of the peripheral cannabinoid receptor CB2, and to pharmaceutical compositions thereof comprising as an active ingredient novel (+) α-pinene derivatives, which are useful for prevention and treatment of autoimmune diseases including but not limited to rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, diabetes mellitus type I, hepatitis, psoriasis, tissue rejection in organ transplants, malabsorption syndromes such as celiac disease, pulmonary diseases such as asthma and Sjögren's syndrome, inflammation including inflammatory bowel disease, pain including peripheral, visceral, neurophathic inflammatory and referred pain, muscle spasticity, cardiovascular disorders including arrhythmia, hypertension and myocardial ischemia, neurological disorders including stroke, migraine and cluster headaches, neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's chorea, prion-associated neurodegeneration, CNS poisoning and certain types of cancer.

Synthesis of compound AH: 2,2-dimethylpropionic acid-4-{4-[1,1-Dimethyl-pentyl]-2,6-dihydroxy-phenyl}-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl methyl ester.

The synthesis of compound **AH** is depicted in scheme 21.

Scheme 21.



4-hydroxymyrtenyl-pivalate was prepared as described in U.S. Patent No. 4,876,276 and 5-(1,1-dimethylpentyl)-resorcinol was prepared as follows. In a 250 ml round bottom flask 100 ml of methanol and 100 ml of THF were added. Then 5.5 g of 3,5-dimethoxy-benzoic acid (0.03 mole) and 1.27 g of lithium hydroxide monohydrate (0.03 mole) were added. Then 10 ml of water was added and the reaction mixture was stirred for 1 hour. The slurry obtained was filtered and evaporated. The residue was titurated with ether and evaporated again to obtained yellowish solid. The solid dried with P_2O_5 under reduced pressure at 60°C. The dried salt of 3,5-dimethoxy lithium benzoate was added to a 250 ml round bottom flask filled with 100 ml of THF. N-butyl lithium (20 ml, 1.7 M, 0.032 mole) was added. The reaction was warmed up to 50°C and stir for two hours. Then the reaction mixture was cooled to room temperature and added dropwise to 250 ml of 1 N HCl. Then Na_2CO_3 was added until pH ~ 11. Then the reaction mixture was extracted 3 times with ether. The combined organic phases were dried over sodium sulfate filtered and evaporated to give orange oil, which crystallized from n-pentane. 2.6 g of compound **12** wherein R is butyl was obtained, with an overall yield of 39%. Compounds **13** and **14** wherein R is butyl were prepared as described in scheme 7. The condensation between 4-hydroxymyrtenyl-pivalate and 5-(1,1-dimethylpentyl)-resorcinol was performed as described for compound E and the yield was 65%.

PHYSIOLOGICAL EXAMPLES

Evaluation of the therapeutic effects of the novel bicyclic CB2 ligands was carried out in a series of experimental systems to support the utility of these drugs as immunomodulatory,

anti-inflammatory, analgesic, neuroprotective and anti-tumoral agents. These effects were evaluated both *in vitro* and *in vivo*, and corroborated utilizing the systems described below. Unless otherwise indicated the test compounds are prepared as follows: for in vitro assays the compounds are first dissolved in DMSO and then stepwise diluted in the assay buffer, generally tissue culture medium, down to a final concentration of 0.1% DMSO. For in vivo assays the test compounds are first diluted in CREMOPHOR EL®:ethanol (70% and 30% w/w respectively) and further diluted 1:20 in physiological buffer, generally saline, to reach the appropriate dose. Thus the vehicle is the original "solvent" diluted in the appropriate buffer.

Example 1

Binding affinity for the CB1 and CB2 receptors.

The CB1 binding assays were performed by testing the ability of the new compounds to displace [^{3}H]CP55940 from the CB1 receptor on membranes derived from hCB1 stably transfected HEK-293 cells (Perkin Elmer/NEN). Membranes were diluted in the assay buffer (50 mM Tris-HCl, 2.5 mM EDTA, 5 mM $MgCl_2$, 1 mg/ml BSA, pH=7.4) to 500 μg protein /ml. 50 μl of diluted membranes (25 μg) were incubated with [^{3}H]CP55940 in the presence or absence of the bicyclic test compounds in a total volume of 0.5 ml. Tested compounds were dissolved in DMSO and diluted in the assay buffer to a final concentration of 0.1% solvent. Control samples were added with identical amount of vehicle. Non-specific binding was measured by the addition of 10 μM of WIN 55212-2. Following 1.5 hours incubation at 30°C reactions were filtered through Whatman 934A/H filters (presoaked with 0.1% Polyethylenimine (PEI)).

The affinities of the novel bicyclic analogs to the CB2 receptor were determined by their ability to displace [^{3}H]WIN 55212-2 from the receptor in membranes derived from hCB2 stably transfected CHO cells (Perkin Elmer/NEN). Membranes were diluted in assay buffer (10 mM HEPES, 1 mM $MgCl_2$, 1mM EDTA, 0.3 mg/ml BSA, pH=7.4) to 500 μg protein/ml. 50 μl of diluted membranes (25 μg) were incubated with 0.8 nM of [^{3}H]WIN 55212-2 in the presence or absence of several concentrations of the bicyclic test compounds in a total volume of 1 ml. Tested compounds were dissolved and diluted as previously described for the hCB1 assay. Non-specific binding was measured by the addition of 10 μM CP 55940. Following 40 minutes incubation at 30°C reactions were filtered as previously described. Filters for all binding assays were counted in a β-counter and log of analog concentration versus % of binding was plotted. IC_{50} values were extrapolated from this plot.

The results of the binding assays are shown in Table 1, which depicts the Structure Activity Relationship (SAR) of the preferred compounds, in terms of their ability to displace [^{3}H]WIN 55212-2 or [^{3}H]CP55940 from CB2 or CB1 binding sites, respectively.

The abbreviations used in Table 1 to define R_2, R_3 and R_4 refer to the following substituents:

DMBP= 1,1-Dimethyl-5-Bromo-Pentyl
DMCP= 1,1-Dimethyl-5-Cyano-Pentyl
DMEP= 1,1-Dimethyl-Ethyl-Phenyl
DMH= 1,1-Dimethyl Heptyl
DMH6= 1,1-Dimethyl Hept-6-ynyl
DMP= 1,1-Dimethyl Pentyl
DMPP= 1,1-Dimethyl-3-Phenyl-Propyl
EMP= 1-Ethyl-1-Methyl-Propyl
MCPE= 1-Methyl-1-(p-Chloro-Phenyl)-Ethyl

The values of IC_{50} reported in table 1 were calculated from graphs such as depicted in Figure 1, which shows the binding of selected bicyclic compounds to the cannabinoid receptors. Binding to CB1 is measured by competitive inhibition of [^{3}H]CP55940 in HEK-293 cells stably transfected with the human CB1 receptor gene. Binding to CB2 is measured by competitive inhibition of [^{3}H]WIN55212-2 in CHO cells stably transfected with the human CB2 receptor gene. Both curves (hCB1 ■ and hCB2 ♦), representing % inhibition as a function of compound concentration, are superimposed in this graph. A- Displays the results obtained with compound A. B- Displays the results obtained with compound B. C- Displays the results obtained with compound J. D- Displays the results obtained with compound L.

TABLE 1. SAR and IC_{50} (nM) of bicyclic compounds of formulae (I) to (III).

COMPOUND	R_1	R_2	R_3	R_4	R_5	CB2 IC_{50}	CB1 IC_{50}	CB2/CB1 affinity ratio
HU-210*						0.35	0.39	1.11
HU-308*		OCH_3	OCH_3	DMH	CH_2OH	13.3	3600	271
A	O	OH	OH	DMH		1	27.6	28
B	O	OCH_3	OCH_3	DMH		45	2800	62
C		OH	OH	DMH	OH	3.5	31	9
D	N-OH	OH	OH	DMH		3.4	93	27
E		OH	OH	DMH6	CH_3	0.783	26	33
F	O	OH	OH	DMH6		0.344	13	38
G	O	OH	OH	DMPP		6.6	563	85
J	O	OH	OH	MCPE		11	659	60
L	O	OH	OH	DMP		3.8	446	117
M	O	OH	OH	EMP		40.8	3900	96
N	O	OH	OH	DMBP		0.36	50	139
P	O	OH	OH	DMCP		1.55	227	146
Q		OH	OH	DMEP	CH_3	12	640	53
R	O	Succinate	OH	DMH		1.2	41	34
S	O	Succinate	Succinate	DMH		1.52	117	77
Y	O	Succinate	OH	DMP		7.4	315	42
Z	O	Fumarate	OH	DMH		1.2	816	656
AH		OH	OH	DMP	$CH_2OC(O)C(CH_3)_3$	42	398	9.5

Compounds with an asterisk do not fall in the definitions of formulae (I) and (II) and are included for comparison only. HU-210 was disclosed in U.S. Patent 5,284,867 and HU-308 was disclosed in international patent application WO 01/32169.